Preimplantation Genetic Diagnosis for Cystic Fibrosis

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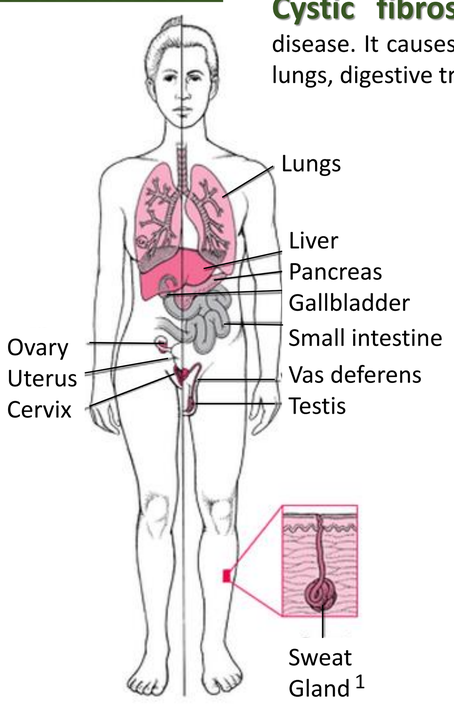
Introduction

Preimplantation genetic diagnosis (PGD), is a technique used to identify genetic defects in embryos created through in vitro fertilization before pregnancy. Since only unaffected embryos are transferred to the uterus for implantation, PGD provides an alternative to prenatal procedures. But it was not until 1989 in London that the first unaffected child was born with a successfully PGD cycle. Even though cystic fibrosis (CF) was the first monogenic disorder to accomplish a successful PGD cycle, it was not until 1992 that they succeeded. Nowadays this technique is practiced worldwide, one of the challenges that PGD had to undertake was that only one cell is available for the diagnosis. The purpose of this review is to understand cystic fibrosis as well as to comprehend the techniques that made PGD possible, through the years up to the present time.

The disease

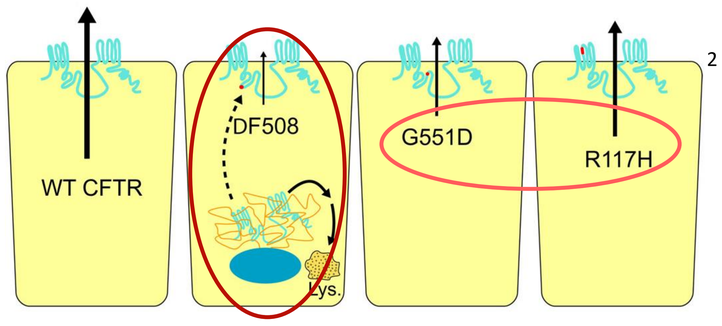


[1]

Cystic fibrosis is an autosomal recessive chronic disease. It causes thick and sticky mucus to build up in the lungs, digestive tract and other areas of the body.

More than 1.900 mutations in the CFTR gene

(*Cystic Fibrosis Transmembrane Conductance Regulator*)



[2]

↑Frequent and ↑deleterious mutation in the Caucasian population

Milder mutations

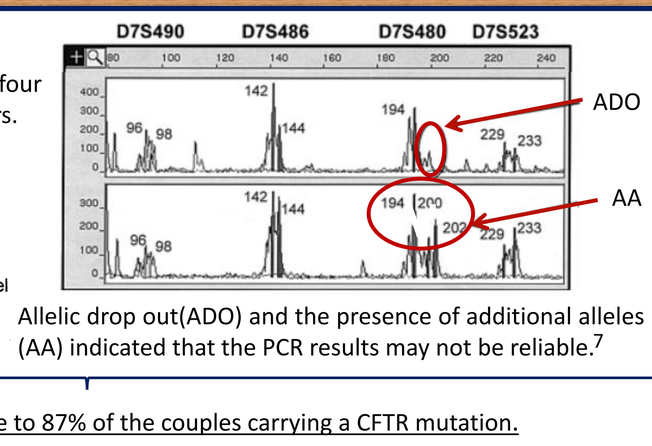
The symptoms and the severity of the disease can vary widely among patients.

CFTR – Related Disorders

Typically: ΔF508 with R117H or IVS8-5T

- Congenital bilateral absence of the vas deferens (CBAVD)
- Acute or recurrent chronic pancreatitis
- Disseminated bronchiectasis

Causes of Variability in Outcomes [3]



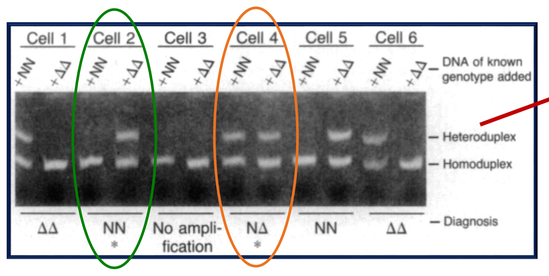
Diagnostic Methods History and Present

1986 The consensus view was that the blastocyst biopsy was the most likely to succeed.

1989 An approach was determined, valuable for couples at risk of transmitting X-linked diseases. → The sex was determined with a Y specific probe.

1990 This year were reported <u>the first established pregnancies using PGD,</u> in two couples known to be at risk of transmitting adrenoleukodystrophy an X-linked mental retardation.

1992 Using <u>the polymerase chain reaction (PCR) with nested primers</u>, a normal fragment of 154pb from the CFTR gene was amplified, this fragment included the ΔF508 region (151pb).



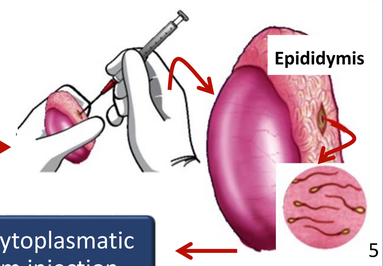
Key of the technique: The migration of the DNA heteroduplex is retarded.[4]

Embryos that could be transferred:
Healthy non carrier embryo.
Healthy embryo (but ΔF508 carrier).

First healthy girl (from embryo 2) born free of Cystic Fibrosis through PGD.

1994 First time with PGD for Cystic Fibrosis..

Men with CF usually have CBAVD → Microsurgical epididymal sperm aspiration (MESA) was performed to obtain spermatozoa → Intracytoplasmatic sperm injection



[5]

1995 There was a need to <u>detect more than 1 mutation</u> from one blastomere...

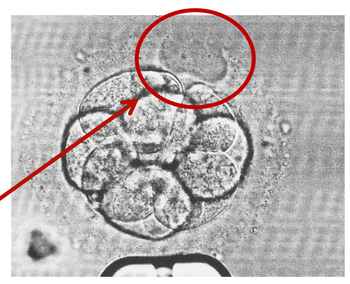
W1282X (W) ΔF508 (Δ)
- Common mutations.
- Severe phenotypes: WW, ΔΔ and WΔ.

→ Two-round PCR amplification → Multiplex PCR → Nested PCR separately (at each locus)

1998 Cell obtaining methodology..

- Aspiration method
- Use of Tyrode's acid solution (reduced embryo viability)

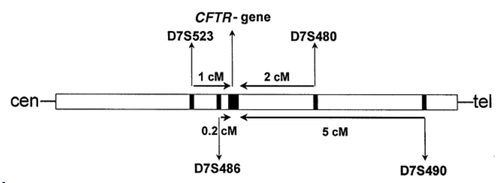
Use of a diode noncontact laser → Quicker, simpler and safer. → Well-defined hole.[6]



2000

<u>Multiplex marker PCR protocol</u>, with four closely linked highly polymorphic markers.

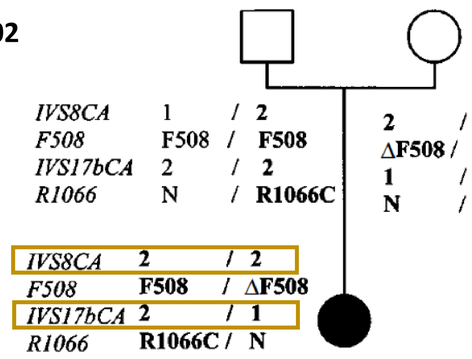
Micro-satellites markers flanking the *CFTR* gene [7]



Allelic drop out(ADO) and the presence of additional alleles (AA) indicated that the PCR results may not be reliable.[7]

This protocol could <u>be applicable to 87% of the couples carrying a CFTR mutation.</u>

2002



In 91% of the cases at least one partner of the couple carries the ΔF508 mutation.

This strategy was based on a multiplex fluorescent PCR co-amplifying the ΔF508 mutation and two CFTR intragenic polymorphic microsatellites (IVS8CA and IVS17bCA).[8]

The use of intragenic markers → ↓The risk of misdiagnosis due to meiotic recombination

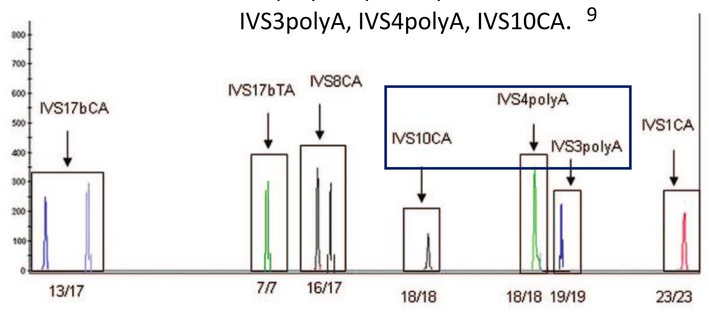
Four intragenic polymorphic repeats identified: IVS1CA, IVS8CA, IVS17bTA and IVS17bCA.

2009

A procedure was validated for the seven microsatellites in a single run, which detects approximately 90% of the mutant alleles.

Consequently, 1 or both causal mutations were unknown in about 20% of the cases.

Three novel polymorphic repeats were identified: IVS3polyA, IVS4polyA, IVS10CA. [9]



2013

In the majority of the cases linkage analysis is performed.

Fluorescent in situ hybridization (FISH) is also used in order to assure no chromosomal abnormalities.

Single cell amplification could be undertook by nested – PCR, fluorescent – PCR, multiplex – PCR or a combination of them.

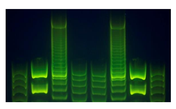
Nowadays the PCR sensitivity is 99.2% and its specificity is 80.9%, however the accuracy is statistically higher when PGD is performed on two cells and multiplex protocols are applied.

Conclusions and future developments

Even though the validity, robustness and high diagnostic value of PCR-based PGD has been demonstrate, the wide range of mutations for CF and CFTR – Related Disorders are still a problem for assuring the identification of both embryonic alleles.

Present time: Molecular methods

Low throughput
Time consuming



Future: Next generation sequencing (NGS) technologies

Diagnostic rate of 98.91%
↓Time ↓Cost

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